

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a nucleic acid selected from the group consisting of
5 a nucleic acid encoding any one of *Blm* open reading frames (ORFs) 8 through 41;

a nucleic acid encoding a polypeptide encoded by any one of *Blm* open reading frames (ORFs) 8 through 41; and

10 a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II and the nucleic acid of a bleomycin-producing organism as a template.

2. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding at least two open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

15 3. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding at least three open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

20 4. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding a C domain lacking one or more His residues of the conserved HHxxxDG active site for transpeptidation.

5. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmI*, *blmII*, and *blmXI*.

25 6. An isolated nucleic acid comprising a nucleic acid encoding a module comprising two or more catalytic domains of a protein encoded by a nucleic acid of a bleomycin gene cluster wherein said catalytic domains are selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain,

an oxidization domain (Ox), a ketoacyl synthase (KS) domain, an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

7. The isolated nucleic acid of claim 6, wherein said nucleic acid comprises a nucleic acid encoding one or more proteins comprising a module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-9, and PKS.

8. The isolated nucleic acid of claim 7, wherein said nucleic acid comprises an open reading frame from SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

9. An isolated nucleic acid comprising a nucleic acid encoding a protein encoded by a gene from a BLM gene cluster.

10. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmI*, *blmII*, and *blmXI*.

11. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmIII*, *blmIV*, *blmV*, *blmVI*, *blmVII*, *blmIX*, and *blmX*.

12. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by *blmVIII*.

13. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid selected from the group consisting of *blmI*, *blmII*, and *blmXI*.

14. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid selected from the group consisting of *blmIII*, *blmIV*, *blmV*, *blmVI*, *blmVII*, *blmIX*, and *blmX*.

15. The nucleic acid of claim 9, wherein said nucleic acid comprises *blmVIII*.

16. An isolated nucleic acid comprising a nucleic acid that encodes a protein comprising at least one catalytic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP)

domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain, an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain, and that hybridizes to a nucleic acid selected from the group consisting of *orf8*, *orf9*, *orf10*, *orf11*,
5 *orf12*, *orf13*, *orf14*, *orf15*, *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*,
orf25, *orf26*, *orf27*, *orf28*, *orf29*, *orf30*, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*,
orf39, *orf40*, and *orf41* under stringent conditions.

17. The nucleic acid of claim ~~16~~, wherein said isolated nucleic acid comprises a nucleic acid encoding a module.

18. The nucleic acid of claim ~~16~~, wherein said isolated nucleic acid comprises a nucleic acid encoding a BLM gene.

19. An isolated nucleic acid comprising a nucleic acid selected from the group consisting of consisting of *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*, *orf15*,
15 *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*, *orf25*, *orf26*, *orf27*, *orf28*, *orf29*,
orf30, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*, *orf39*, *orf40*, and *orf41*, or an allelic variant thereof.

20. The nucleic acid of claim ~~19~~, wherein said nucleic acid comprises a nucleic acid that is a single nucleotide polymorphism (SNP) of a nucleic acid selected from the group consisting of consisting of *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*,
20 *orf15*, *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*, *orf25*, *orf26*, *orf27*, *orf28*,
orf29, *orf30*, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*, *orf39*, *orf40*, and *orf41*.

21. An isolated gene cluster comprising open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin.

22. An isolated multi-functional protein complex comprising both a
25 polyketide synthase (PKS) and a peptide synthetase (NRPS).

23. An isolated nucleic acid encoding a multi-functional protein complex comprising both a polyketide synthase (PKS) and a peptide synthetase (NRPS).

24. An isolated polypeptide comprising a catalytic domain encoded by a nucleic acid of a bleomycin gene cluster wherein said nucleic acid comprises a nucleic acid selected from the group consisting of

a nucleic acid encoding any one of *Blm* open reading frames (ORFs) 8 through 41; and

a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II.

25. The polypeptide of claim 25, wherein said polypeptide comprises an enzymatic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain, an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

26. The polypeptide claim 25, wherein the nucleic acid of a bleomycin gene cluster comprises a nucleic acid encoding at least two open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

27. The polypeptide claim 25, wherein said nucleic acid of a bleomycin gene cluster comprises a nucleic acid encoding at least three open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

28. The polypeptide claim 25, wherein said polypeptide comprises a C domain lacking one or more His residues of the conserved HHxxxDG active site for transpeptidation.

29. The polypeptide claim 25, wherein said polypeptide is a polypeptide encoded by a gene selected from the group consisting of *blmI*, *blmII*, and *blmXI*.

30. An isolated polypeptide comprising a module comprising two or more catalytic domains of a protein encoded by a nucleic acid of a bleomycin gene cluster wherein said catalytic domains are selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an

oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

31. The polypeptide of claim 30, wherein said polypeptide comprises a module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4,
5 NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS.

32. An isolated polypeptide encoded by a gene from a BLM gene cluster.

33. The polypeptide of claim 32, wherein polypeptide is encoded by a gene selected from the group consisting of *blmI*, *blmII*, and *blmXI*.

34. The polypeptide of claim 32, wherein said nucleic acid comprises a
10 nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmIII*, *blmIV*, *blmV*, *blmVI*, *blmVII*, *blmIX*, and *blmX*.

35. The polypeptide of claim 32, wherein polypeptide is encoded by
blmVIII.

36. An isolated polypeptide comprising a module wherein said module is
15 specifically bound by an antibody that specifically binds to a BLM module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS.

37. The polypeptide of claim 36, wherein said polypeptide is specifically bound by an antibody that specifically binds to a polypeptide encoded by a gene selected
20 from the group consisting of of *blmI*, *blmII*, *blmXI*, *blmIII*, *blmIV*, *blmV*, *blmVI*, *blmVII*, *blmIX*, *blmX*, and *blmVIII*.

38. An isolated polypeptide comprising a polypeptide encoded an open reading frame of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, or an allelic variant thereof.

39. The polypeptide of claim 38, wherein said nucleic acid comprises a
25 single nucleotide polymorphism (SNP) of an open reading of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

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40. An expression vector comprising a nucleic acid of any one of claims 1 through 23.

41. A host cell transformed with an expression vector of claim 40.

42. The host cell of claim 41, wherein said cell is transformed with an exogenous nucleic acid comprising a gene cluster encoding polypeptides sufficient to direct the assembly of a bleomycin or bleomycin analog.

43. The cell of claim 41, wherein said cell is a bacterial cell.

44. The cell of claim 43, wherein said cell is a *Streptomyces* cell.

45. The cell of claim 41, wherein said cell is a eukaryotic cell.

46. A method of chemically modifying a biological molecule, said method comprising contacting a biological molecule that is a substrate for a polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames with the polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames, whereby said polypeptide chemically modifies said biological molecule.

47. The method of claim 46, wherein said method comprising contacting said biological molecule with at least two different polypeptides encoded by *blm* gene cluster open reading frames.

48. The method of claim 46, wherein said method comprising contacting said biological molecule with at least three different polypeptides encoded by *blm* gene cluster open reading frames.

49. The method of claim 46, wherein said contacting is in a host cell.

50. The method of claim 49, wherein said host cell is a bacterium.

51. The method of claim 46, wherein said contacting *ex vivo*.

52. The method of claim 46, wherein said biological molecule is an endogenous metabolite produced by said host cell.

53. The method of claim 46, wherein said biological molecule is an exogenous supplied metabolite.

54. The method of claim 46, wherein said host cell is a eukaryotic cell.

55. The method of claim 54, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, a yeast cell, a plant cell, a fungal cell, and an insect cell.

56. The method of claim 46, wherein said biological molecule is an amino acid and said polypeptide is a peptide synthetase.

57. The method of claim 46, wherein said polypeptide is a methyl transferase.

58. A method of coupling a first amino acid to a second amino acid, said method comprising contacting the first and second amino acid with a recombinantly expressed bleomycin nonribosomal peptide synthetase (NRPS).

59. The method of claim 64, wherein said NRPS is selected from the group consisting of NRPS-5, NRPS-4, NRPS-3, NRPS-9, NRPS-8, and NRPS-7.

60. The method of claim 64, wherein said NRPS is selected from the group consisting of NRPS-6, NRPS-2, NRPS-1, and NRPS-0.

61. The method of claim 64, wherein said contacting is in a host cell.

62. A method of coupling a first fatty acid to a second fatty acid, said method comprising contacting the first and second fatty acids with a recombinantly expressed bleomycin polyketide synthase (PKS).

63. The method of claim 62, said contacting is in a host cell.

64. A method of producing a bleomycin or bleomycin analog, said method comprising:

providing a cell transformed with an exogenous nucleic acid comprising a bleomycin gene cluster encoding polypeptides sufficient to direct the assembly of said bleomycin or bleomycin analog;

culturing the cell under conditions permitting the biosynthesis of bleomycin or bleomycin analog; and

isolating said bleomycin or bleomycin analog from said cell.

65. An isolated nucleic acid comprising a nucleic acid encoding a phosphopantetheinyl transferase said nucleic acid encoding a phosphopantetheinyl transferase being selected from the group consisting of:

a nucleic acid encoding the protein encoded by the nucleic acid of SEQ ID NO:3;

a nucleic acid amplified by polymerase chain reaction (PCR) using primers that specifically amplify ORF 41 (primers: SEQ ID NO:71 and SEQ ID NO:72) and *Streptomyces* nucleic acid as a template;

a nucleic acid encoding a polypeptide having phosphopantetheinyl transferase activity where said nucleic acid specifically hybridizes to the nucleic acid of SEQ ID NO: 3 under stringent conditions.

66. The nucleic acid of claim 65, said nucleic acid comprising a nucleic acid of SEQ ID NO:3.

67. A polypeptide comprising a phosphopantetheinyl transferase encoded by SEQ ID NO:3.

68. A vector comprising the nucleic acid of claim 66.

69. A cell transfected with the vector of claim 68.

70. A method of converting an apo-carrier protein to a holo-carrier protein comprising reacting said apo-carrier protein with a recombinant phosphopantetheinyl transferase encoded by SEQ ID NO:3 and coenzyme A thereby producing a holo-carrier protein.

71. A cell comprising a modified bleomycin gene cluster nucleic acid, said cell producing elevated amounts of bleomycin as compared to the wild type cell.

72. The cell of claim 71, wherein said cell overexpresses a resistance gene from the bleomycin gene cluster.

73. The cell of claim 72, wherein said resistance gene is a gene listed in Table III.

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